L6 ANSWER 5 OF 5 CA COPYRIGHT 2000 ACS DUPLICATE 5

ACCESSION NUMBER: 107:148280 CA

TITLE: Localization of stx, a determinant essential for high-level production of Shiga toxin by Shigella dysenteriae serotype 1, near pyrF and generation of stx transposon mutants

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CORPORATE SOURCE: Dep. Med. Biochem., Univ. Geneva, Geneva, CH-1211/4, Switz.

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AB Hfr strains of S. dysenteriae serotype 1 were constructed by transient integration of an RP4 plasmid deriv. carrying transposon Tn501 into the Shigella chromosome through Tn501-mediated cointegration. The Hfr strain were mated with Escherichia coli K-12 recipients carrying various auxotrophic markers, and E. coli recombinants which had received prototrophic Shigella genes were selected. Some of the E. coli transconjugants produced high levels of a cytotoxin which was neutralized by both polyclonal and monoclonal anti-Shiga toxin sera. The determinant for Shiga toxin prodn., designated stx, was first transferred to E. coli K-12 and then mapped by Hfr crosses to the trp-pyrF region located at 30 min on the E. coli chromosome. Bacteriophage P1-mediated transduction anal. of stx gave the following gene order: trp-pyrF-stx. The level of Shiga toxin prodn. in E. coli Stx+ transconjugates and transductants was as high as that of the parental S. dysenteriae 1 strain. Stx- mutants of an Stx+ E. coli transductant were generated by random in-vivo insertion mutagenesis with a Tn10 deriv. transposon, Tn-mini-kan, followed by P1 cotransduction of the kanamycin(kan)-resistance and PyrF+ markers into a pyrF Stx+ E. coli K-12 recipient. One stx::Tn-mini-kan transposon mutation was transferred by P1 transduction from this E. coli Stx- mutant to an E. coli K-12 Hfr strain and, in turn, transferred by conjugation to the original S. dysenteriae 1 strain plus two others. All kanamycin-resistant recombinants of S. dysenteriae 1 had lost their ability to produce high levels of Shiga toxin. A gene that specifies high-level Shiga toxin prodn. is, thus, located near pyrF on the chromosome of S. dysenteriae 1. Stx- mutants of S. dysenteriae 1 exhibited full virulence in the Sereny test.

L7 ANSWER 1 OF 1 BIOSIS COPYRIGHT 2000 BIOSIS
ACCESSION NUMBER: 1989:72660 BIOSIS
DOCUMENT NUMBER: BA87:37058
TITLE: GENETICS AND REGULATION OF ENTEROBACTIN GENES IN **SHIGELLA**-FLEXNERI.
AUTHOR(S): SCHMITT M P; PAYNE S M
CORPORATE SOURCE: DEP. MICROBIOL., UNIV. TEXAS AT AUSTIN, AUSTIN, TEXAS 78712.
SOURCE: J BACTERIOL, (1988) 170 (12), 5579-5587.
CODEN: JOBAAY. ISSN: 0021-9193.
FILE SEGMENT: BA; OLD
LANGUAGE: English

AB Although **Shigella** flexneri possesses the genes for two siderophore systems, enterobactin and aerobactin, the enterobactin system is only rarely utilized. To investigate the regulation of enterobactin expression in S. flexneri, all of the genes specifically required for synthesis and transport of enterobactin were cloned from both an expressing (Ent+) strain. Notable differences between the cloned genes included endonuclease restriction site changes and the presence of an IS1 element in the Ent- DNA. Southern hybridization revealed that this IS1 element, present at the 3' end of the entF gene, is conserved at this location in different strains and serotypes of Ent- S. flexneri. The Ent- cloned genes were tested for their ability to complement the defect in 11 different Escherichia coli enterobactin mutants. The Ent- genes fully complemented nine mutants but failed to complement the entF mutant AN117 and only partially complemented the entE mutant AN93. Whole-cell RNA isolated from E. coli and the **Shigella** strains was hybridized to 32P-labeled DNA containing the entB gene or a fragment carrying a portion of the entF gene. E. coli and the Ent+ **Shigella** strains exhibited depression of transcription of these genes in low-iron media. Transcription in the Ent strain remained repressed regardless of iron concentration. Expression of the entB and entF genes was also examined in an Ent- **Shigella** fur mutant. Expression of entF was only partially depressed and entB remained fully repressed at all iron concentrations, suggesting that factors other than Fur are responsible for the repression of these enterobactin genes in the Ent- **Shigella** strains.